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CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. IV.

NOTE ON SOME MYXOSPORIDIA FROM CERTAIN FISH IN THE VICINITY OF WOODS HOLE *

Rokusaburo Kudo

The following is a brief summary of observations made on Cnidosporidian parasites of certain fish in the vicinity of Woods Hole in the summer of 1916. Six species of the parasites were found in eighteen species of fish, chiefly of salt water, which came under my observation. The names of the fish, together with those of the parasites, are shown in the following table.

TABLE 1

Species	Number of Fish Examined	Number of Fish Having Parasites	Organs Harboring Parasites	Parasites
Anguilla chrysypa.........	3			
Bothus maculatus.........	1			
Centropristes striatus.....	2			
Esox. sp.	3			
Fundulus heteroclitis...... Fundulus majalis	78	34	Gill	Myxosoma funduli n. sp.
Menidia sp.	3			
Morone americana.........	12			
Mustelus canis.............	1			
Paralichthys dentatus.....	15	7	Gallbladder	Ceratomyxa drepanosettae Awerinzew
Perca flavescens...........	30	1	Spleen	Myxobolus pyriformis Thélohan
Phycis tenuis..............	1	1	Gallbladder	Small number of Myxosporidia in vegetative stage
Prionolus carolinus........	12			
Raia erinacea..............	1	1	Gallbladder	Small number of Myxosporidia in vegetative stage
Rhombus tricanthus.......	11			
Scomber scrombrus*.......	20			
Stenotomus chrysops......	14	4	Gallbladder	Ceratomyxa sp.
Tautaga omitis............	5			
Tautogolabrus adspersus	10			

* It is quite remarkable to note that Fantham and Porter (1912) found 4 out of 25 fish from French waters which harbored a Myxosporidium.

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* From the Marine Biological Laboratory, Woods Hole, and the Laboratories of the Rockefeller Institute for Medical Research.

The fish were brought into the laboratory alive. A study of the external characters and the branchial lamellae under a dissecting microscope was followed by a microscopical examination of the liver, spleen, kidney, urinary bladder and gallbladder. The dark field microscope was used to a greater extent than the ordinary microscope.

For the extrusion of the polar filament, I have been using in my recent work chiefly mechanical pressure and alkali. Perhydrol, which was proved to be an energetic reagent for *Nosema bombycis* (1918) was used on the spores of various Cnidosporidia with satisfactory results. Fixing and staining were usually done as described in previous papers, with a few modifications.

Myxosoma funduli nov. spec.

Habitat: The branchial lamellae and connective tissue of the gill-filament of *Fundulus heteroclitis* and *F. majalis.*

The cysts distinguish themselves from the gill as small white spots attached to the surface of the gill (Fig. 2). In the case of heavy infection, it is not uncommon to find many cysts in one line parallel to the free end of the gill (Fig. 1), similar to those observed by Müller (1841, Fig. 7).

The form of the cysts is usually spherical (Fig. 2), the average diameter being about 150μ. The largest one, however, which was encountered, measured 360μ by 264μ. All the cysts found in sections were in the final stage of development, so that young and matured spores only could be observed.

The spore is pyriform (Text figure A, Figs. 3-7). The spore coat, which is usually uniform in thickness, shows seven to ten markings on the posterior half of its surface (Text fig. A, *a, d,* and Fig. 3). A process similar to that of *Myxosoma dujardini* and *Myxobolus toyamai* was found occasionally (Text fig. A, *c*). The spore-membrane is composed of two halves, the line of junction being parallel to the line connecting the two polar capsules (Text fig. A, *b*). It is straight and thickened a little along the line of junction. The average dimensions of the spores are 14μ in length, 8μ in breadth, and 6μ in thickness. The optical cross-section, therefore, is always oval (Fig. 4).

Two polar capsules and the sporoplasm are found in the sporecoat. The former is pyriform in shape, with dimensions of 8μ in length and 2μ in width. The length of the polar filament is 38 to 42μ. Figure 6 shows one of the filaments extruded under mechanical pressure, though not at its full length. Pressure also changes the form of the spore. For this reason, perhydrol is much to be preferred to pressure, as may be seen from a comparison of figures 5 and 7.

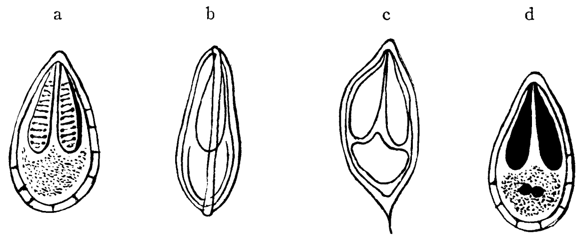
The sporoplasm is granular in structure. Two nuclei were detected in stained preparations (Text fig. A, *d*). In fresh preparations the faint outline of a vacuole-like structure was occasionally observed in the sporoplasm. No iodophile vacuole, however, appears when the spore is treated with iodine water, iodine alcohol, or Lugol's solution.

Hahn (1913, 1917) described *Myxobolus musculi* found in the integument, muscles, and gills of fish of the same species in the same locality. During my examination I failed to encounter any parasites in the integument.

The spore of *Myxobolus musculi,* according to Hahn, is 14.3μ long and 6μ wide and has two polar capsules (2 by 0.5μ). Moreover, it has an iodophile vacuole in the sporoplasm and has no marking on the coat. He proved by an infection experiment that the Myxobolus found in the gill and that found in the muscle of Fundulus were the same, though he seems to have noticed some different characters, as for example, a difference in the form of the parasite.

Linton described a Myxosporidian found in the integument of *Cyprinodon variegatus* from the same locality, which was also observed and described by Gurley as *Myxobolus lintoni* Gurley. The form of the spore of the species mentioned is somewhat similar to the present form, the latter, however, being narrower at the anterior half than the former. The dimensions given by Linton and Gurley are: length 13.9μ, breadth 11μ and thickness 8μ. Gurley detected an iodophile vacuole in the sporoplasm; this was not the case with the form in question.

TEXT FIG. A



The absolute absence of any iodophile vacuole in the present species leads me to describe it as belonging to the genus Myxosoma (Thélohan), which Gurley included in the genus Chloromyxum.

Of the two species hitherto known, *Myxosoma ambiguum* Thélohan is quite different from the one in question. *Myxosoma dujardini* has a spore similar in size and form to the present form but without the marking on the coat. Moreover, the spore-coat of the species abovementioned is typically more thickened at the anterior end than in the one I observed. There is also a difference between the polar filaments of the two forms.

It is apparent from my description of the species that it seems to be the same as that described by Hahn found in the gill of fish of the same kind. I propose for it, in accordance with my observations, the name *Myxosoma funduli.*

Ceratomyxa drepanopsettae Awerinzew

Habitat: The gallbladder of *Paralichthys dentatus.*

Seven out of fifteen individuals of about 30 cm. in length harbored the parasite.

Fantham and Porter (1912) made an observation on the effect of Myxosporidia upon the bile and gallbladder of fish. My observations are somewhat similar to the results mentioned by them. The wall of the infected gallbladder was usually thick and opaque. In this connection it may be interesting to mention one of my previous papers (1916). In the case of *Acheilognathus lanceolatum,* the wall of the gallbladder, highly infected with *Zschokkella acheilognathi* Kudo, was found rather thinner and more transparent in some cases than the normal ones, so that the parasites in the bladder and the duct could easily be seen from the outside. On the other hand, it was not rare to find an opaque gallbladder without any parasite in it. In two instances the gallbladder was only about two-thirds of that of other infected fish of similar length.

The bile of the normal fish was clear and greenish, while that of the infected ones was pale yellowish with a large amount of the floating mass, made up chiefly of degenerating epithelium, in which the parasites were found. The vegetative form found in the bile varies greatly in size and form, being sometimes slender and somewhat angular with three or four long fine pseudopodia, and sometimes flat and round, with or without pseudopodia (Figs. 8 to 11). It is not rare, however, to see specimens with a number of fine pseudopodia on all sides of the body (Fig. 8). Some of the psuedopodia attain a length of 30μ. Under the dark field microscope, the form changes very rapidly, probably on account of pressure and temperature. The parasites assume a round shape, withdrawing the pseudopodia (Figs. 10 and 11). A large number of very coarse granules fill up the protoplasm very thickly in almost all cases (Figs. 9, 10 and 11), so that differentiation of the protoplasm into ectoplasm and endoplasm could only be made in very young specimens.

Spores were seldom found in the bile when examined in a fresh condition. It was found, however, that if the bile was kept in small tubes in a refrigerator at about 5 to 15° C. for two or three days, rapid spore-formation took place. These are shown in figures 12 to 14. So far as I could discover, it is always disporous. The dimensions of the spore are: length, 8 to 10μ, breadth 64μ. The round polar capsules have a diameter of about 6μ. Owing to the scarcity of spores, my observation lacks details.

Of all the Ceratomyxa described up to the present time, *Ceratomyxa drepanopsettae* Awerinzew is nearest like the one under discussion. Awerinzew described the species as found in the gallbladder of *Drepanopsetta platessoides.* Later it was found by Auerbach in the gallbladder of *Pleuronectes platessa, P. flesus, Hippoglossus vulgaris,* and *Hippoglossoides limandoides.*

The dimensions are not given by Awerinzew. But the nuclear change in the vegetative form of his illustrations is very much like that of the present species. The probable dimensions of the spore given by Auerbach are about 12 to 14μ in length and 56μ in width, the diameter of the polar capsule being about 4 to 6μ. The form of the spores illustrated by both authors is exactly like the present form. I conclude, therefore, that the two forms are identical.

Myxobolus sp.

Habitat: Spleen of *Perca flavescens.* Only one case of slight infection was encountered.

A very small number of isolated spores were found both in smear and section preparations. The form of the spore is ovoidal, attenuated at the anterior end. The coat is uniformly thick, having one polar capsule and a sporoplasm in which an iodophile vacuole could easily be detected. Two nuclei of the same size (about 2μ) were usually present in the sporoplasm. The dimensions are as follows: Length and breadth of the spore 18 to 20μ and 8μ, respectively. The polar capsule is 7 to 9μ long and 3 to 6μ wide.

The species in question is probably identical with *Myxobolus pyriformis* Thélohan described by him as found in the branchiae and spleen of *Tinca tinca* and kidney of *Misgurnus fossilis.*

I want to express my thanks to Dr. Noguchi for allowing me to carry on these observations in his laboratory.

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EXPLANATION OF PLATES

All the figures were photographed from fresh preparations under dark field illumination.

PLATE I. *Myxosoma funduli* n. sp.

Figs. 1 and 2. The infected gills.

Fig. 3. A group of spores.

Fig. 4. Optical cross section of a spore.

Figs. 5 and 6. Spores treated with perhydrol, showing the extruded filaments.

Fig. 7. A mechanically pressed spore with one of the filaments extruded.

PLATE II. *Ceratomyxa drepanopsettae* Awerinzew

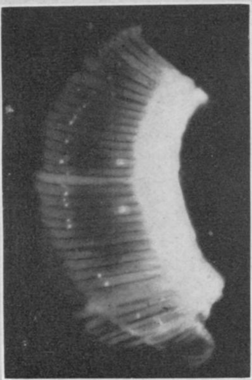
Fig. 8. A relatively young vegetative form with fine pseudopodia.

Fig. 9. A large form, showing the outline of the nuclei and the granules.

Fig. 10. A myxosporidium with a pseudopodium at one end of the body.

Fig. 11. The same specimen after 20 minutes, withdrawing the pseudopodium.

Figs. 12-14. Spores found in the bile after it had been kept in the refrigerator for three days.



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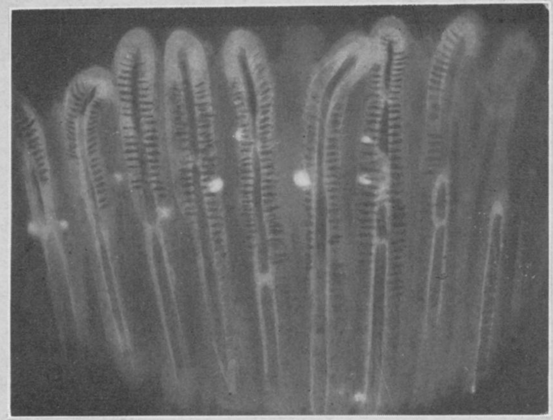

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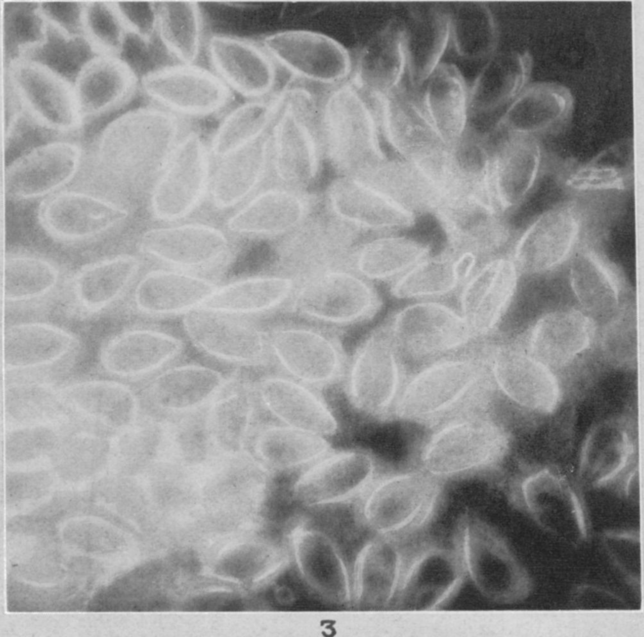

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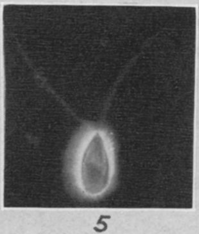

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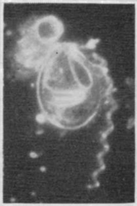

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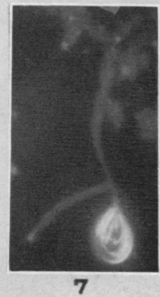

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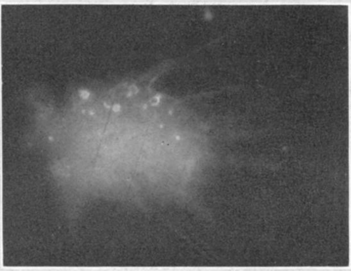

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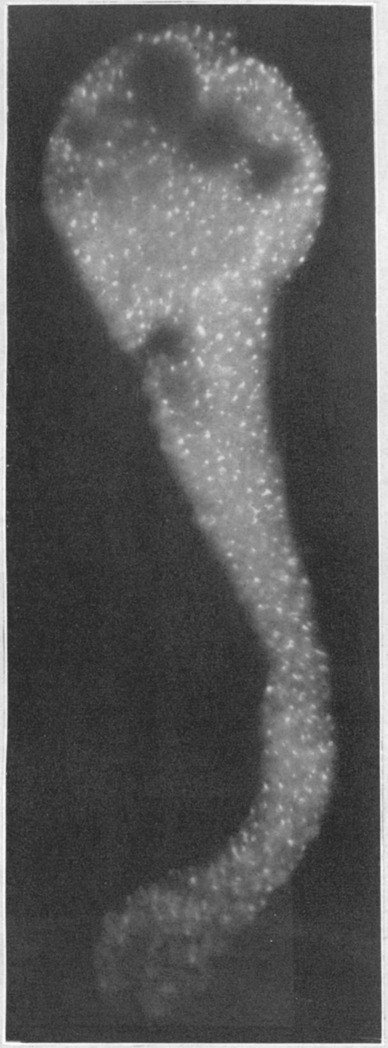

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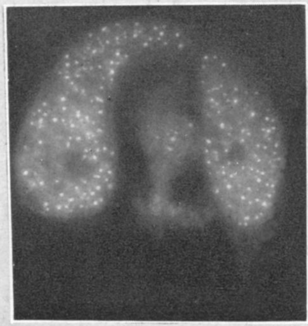

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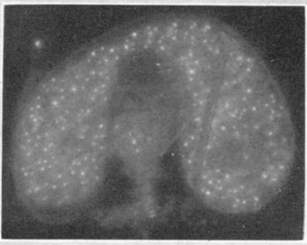

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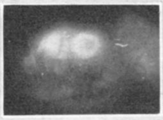

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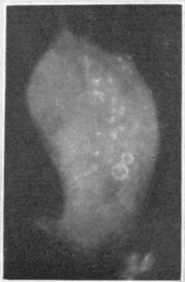

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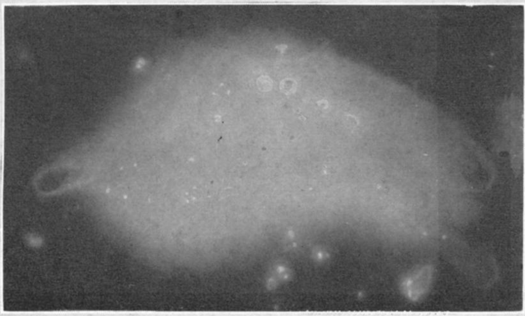

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PLATE II